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## Example 1: Cloning and expression of the hepatitis C virus E1 protein

## 1. Construction of vaccinia virus recombination vectors

The pgptATA18 vaccinia recombination plasmid is a modified version of pATA18 (Stunnenberg et al., 1988) with an additional insertion containing the <u>E. coli</u> xanthine guanine phosphoribosyl transferase gene under the control of the vaccinia virus 13 intermediate promoter (Figure 1). The plasmid pgsATA18 was constructed by inserting an oligonucleotide linker with SEQ ID NO 1/94, containing stop codons in the three reading frames, into the Pst I and HindIII-cut pATA18 vector. This created an extra Pac I restriction site (Figure 2). The original HindIII site was not restored.

Oligonucleotide linker with SEQ ID NO 1/94:

15 3' G GCATGC AAGCTT AATTAATT 3
ACGTC CGTACG TTCGAA TTAATTAA TCGA 5
PStl Sphl Hindill Pac I (Hindill)

In order to facilitate rapid and efficient purification by means of Ni<sup>2+</sup> chelation of engineered histidine stretches fused to the recombinant proteins, the vaccinia recombination vector pMS66 was designed to express secreted proteins with an additional carboxy-terminal histidine tag. An oligonucleotide linker with SEQ ID NO 2/95, containing unique sites for 3 restriction enzymes generating blunt ends (Sma I, Stu I and PmI I/Bbr PI) was synthesized in such a way that the carboxy-terminal end of any cDNA could be inserted in frame with a sequence encoding the protease factor Xa cleavage site followed by a nucleotide sequence encoding 6 histidines and 2 stop codons (a new Pac I restriction site was also created downstream the 3'end). This oligonucleotide with SEQ ID NO 2/95 was introduced between the Xma I and Pst I sites of pgptATA18 (Figure 3).

Oligonucleotide linker with SEQ ID NO 2/95:

XmaI

PstI

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FIGS. 35B-1 to 35B-8: Antibody levels to the different HCV antigens (NS4, NS5, E1 and E2) for NR and LTR followed during treatment and over a period of 6 to 12 months after treatment determined by means of the LIAscan method. The avergae vallues are indicated by the curve with 5 the open squares.

FIGS. 36A and 36B: Average E1 antibody (E1Ab) and E2 antibody (E2Ab) levels in the LTR and NR groups.

FIGS. 37A-D: Averages E1 antibody (E1Ab) levels for non-responders (NR) and long term responders (LTR) for type 1b and type 3a.

FIG. 38: Relative map positions of the anti-E2 monoclonal antibodies.

FIG. 39: Partial deglycosylation of HCV E1 envelope protein. The lysate of vvHCV10A-infected RK13 cells were incubated with different concentrations of glycosidases according to the manufacturer's instructions. Right panel: 20 Glycopeptidase F (PNGase F). Left panel: Endoglycosidase H (Endo H).

FIG. 40: Partial deglycosylation of HCV E2 envelope proteins. The lysate of vvHCV64-infected (E2) and vvHCV41-infected (E2s)RK13 cells were incubated with different concentrations of Glycopeptidase F (PNGase F) according to the manufacturer's instructions.

FIG. 41: In vitro mutagenesis of HCV E1 glycoproteins. Map of the mutated sequences and the creation of new restriction sites.

FIG. 42A: In vitro mutagenesis of HCV E1 glycoprotein (part 1). First step of PCR amplification.

FIG. 42B: In vitro mutagensis of HCV E1 glycoprotein 3 (part 2). Overlap extension and nested PCR.

FIG. 43: In vitro mutagesesis of HCV E1 glycoproteins. Map of the PCR mutated fragments (GLY-# and OVR-#) synthesized during the first step of amplification.

FIG. 44A: Analysis of E1 glycoprotein mutants by Western blot expressed in HeLa (left) and RK13 (right) cells. Lane 1: wild type VV (vaccinia virus), Lane 2: original E1 protein (vvHCV-10A), Lane 3: E1 mutant Gly-1 (vvHCV-81), Lane 5: E1 mutant Gly-3 (vvHCV-83), Lane 6: E1 mutant Gly-4 (vvHCV-84), Lane 7: E1 mutant Gly-5 (vvHCV-85), Lane 8: E1 mutant Gly-6 (vvHCV-86).

FIG. 44B: Analysis of E1 glycosylation mutant vaccinia viruses by PCR amplification/restriction. Lane 1: E1 (vvHCV-10A), BsoE I, Lane 2: E1.GLY-1 (vvHCV-81), BspE I, Lane 4: E1 (vvHCV-10A), Sac I, Lane 5: E1.GLY-2 (vvHCV-82), Sac I, Lane 7: E1 (vvHCV-10A), Sac I, Lane 8: E1.GLY-3 (vvHCV-83), Sac I, Lane 10: E1 (vvHCV-10A), Stu I, Lane 11: E1.GLY-4 (vvHCV-84), Stu I, Lane 13: E1 (vvHCV-10A), Sma I, Lane 14: E1.GLY-5 (vvHCV-85), Sma I, Lane 16: E1 (vvHCV-10A), Stu I, Lane 17: E1.GLY-6 (vvHCV-86), Stu I, Lane 3-6-9-12-15: Low Molecular Weight Marker, pBluescript SK+, Msp I.

FIG. 45: SDS polyacrylamide gel electrophoresis of recombinant E2 expressed in *S. cerevisiae*. Innoculates were grown in leucine selective medium for 72 hrs. and diluted ½1s in complete medium. After 10 days of culture at 28° C., medium samples were taken. The equivalent of 200 µl of 6 culture supernatant concentrated by speedvac was loaded on the gel. Two independent transformants were analysed.

FIG. 46: SDS polyacrylamide gel electrophoresis of recombinant E2 expressed in a glycosylation deficient S. cerevisiae mutant. Innoculae were grown in leucine selective medium for 72 hrs. and diluted  $\frac{1}{15}$  in complete medium. After 10 days of culture at 28° C., medium samples were taken. The equivalent of 350  $\mu$ l of culture supernatant, concentrated by ion exchange chromatography, was loaded on the gel.

Table 1: Features of the respective clones and primers used for amplification for constructing the different forms of the E1 protein as despected in Example 1.

Table 2: Summary of Anti-E1 tests

Table 3: Synthetic peptides for competition studies

Table 4: Changes of envelope antibody levels over time.

Table 5: Difference between LTR and NR

Table 6: Competition experiments between murine E2 monoclonal antibodies

Table 7: Primers for construction of E1 glycosylation mutants

Table 8: Analysis of E1 glycosylation mutants by ELISA

## **EXAMPLE 1**

Cloning and Expression of the Hepatitis C Virus E1 Protein

1. Construction of Vaccinia Virus Recombination vectors

The pgptATA18 vaccinia recombination plasmid is a modified version of pATA18 (Stunnenberg et al, 1988) with an additional insertion containing the *E. coli* xanthine guanine phosphoribosyl transferase gene under the control of the vaccinia virus 13 intermediate promoter (FIG. 1). The plasmid pgsATA18 was constructed by inserting an oligonucleotide linker with SEQ ID NO 1/94, containing stop codons in the three reading frames, into the Pst I and HindIII-cut pATA18 vector. This created an extra Pac I restriction site (FIG. 2). The original HindIII site was not restored.

Oligonucleotide linker with SEQ ID NO 1/94: 5' G GCATGC AAGCTT AATTAATT

3' ACGTC CGTACG TTCGAA TTAATTAA TCGA 5'
Pst1 Sph1 HindIII Pac I (HindIII)

In order to facilitate rapid and efficient purification by means of Ni<sup>2</sup>- chelation of engineered histidine stretches fused to the recombinant proteins, the vaccinia recombination vector pMS66 was designed to express secreted proteins with an additional carboxy-terminal histidine tag. An oligonucleotide linker with SEQ ID NO 2/95, containing unique sites for 3 restriction enzymes generating blunt ends (Sma, Stu I and Pmll/BbrPl) was synthesized in such a way that the carboxy-terminal end of any cDNA could be inserted in frame with a sequence encoding the protease factor Xa cleavage site followed by a nucleotide sequence encoding 6 histidines and 2 stop codons (a new Pac I restriction site was also created downstream the 3' end). This oligonucleotide

ontent of Al

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with SEQ ID NO 2/95 was introduced between the Xma I and Pst I sites of pgptATA18 (FIG. 3).

Oligonucleotide linker with SEQ ID NO 2/95:

was not completely included in construct pvHCV-38, a larger E1 region lacking hydrophobic domain I was isolated from the pvHCV-37 plasmid by EcoRl/Bam HI cleavage and

Oligonucleotide linker with SEQ ID NO 2/95:

### EXAMPLE 2

## Construction of HCV Recombinant Plasmids

2.1. Constructs Encoding Different Forms of the E1 <sub>15</sub> Protein

Polymerase Chain Reaction (PCR) products were derived from the serum samples by RNA preparation and subsequent reverse-transcription and PCR as described previously (Stuyver et al., 1993b). Table 1 shows the features of the 20 respective clones and the primers used for amplification. The PCR fragments were cloned into the Sma I-cut pSP72 (Promega) plasmids. The following clones were selected for insertion into vaccinia reombination vectors: HCCl9A(SEQ ID NO 3), HCCl1 OA (SEQ ID NO 5), HCCl11A (SEQ ID NO 7), HCCl12A (SEQ ID NO 9), HCCl13A (SEQ ID NO 11), and HCCl17A (SEQ ID NO 13) as depicted in FIG. 21. cDNA fragments containing the E1-coding regions were cleaved by EcoRI and HindIII restriction from the respective pSP72 plasmids and inserted into the EcoRl/Hindll-cut 30 pgptATA-18 vaccinia recombination vector (described in example 1), downstream of the 11K vaccinia virus late promoter. The respective plasmids were designated pvHCV-9A, pvHCV-10A, pvHCV-11A, pvHCV-12A, pvHCV-13A and pvHCV-17A, of which pvHCV-11A is shown in FIG. 4. 35

2.2. Hydrophobic Region E1 Deletion Mutants

Clone HCCl37, containing a deletion of codons Asp264 to Val287 (nucleotides 790 to 861, region encoding hydrophobic domain 1) was generated as follows: 2 PCR fragments were generated from clone HCCl10A with primer sets 40 HCPr52 (SEQ ID NO 16)/HCPr107 (SEQ ID NO 19) and HCPr108 (SEQ ID NO 20)/HCPR54 (SEQ ID NO 18). These primers are shown in FIG. 21. The two PCR fragments were purified from agarose gel after electrophoresis and 1 ng of each fragment was used together as template for 45 PCR by means of primers HCPr52 (SEQ ID NO 16) and HCPr54 (SEQ ID NO 18). The resulting fragment was cloned into the Sma I-cut pSP72 vector and clones containing the deletion were readily identified because of the deletion of 24 codons (72 base pairs). Plasmid 50 pSP72HCC137 containing clone HCC137 (SEQ ID 15) was selected. A recombinant vaccinia plasmid containing the full-length E1 cDNA lacking hydrophobic domain I was constructed by inserting the HCV sequence surrounding the deletion (fragment cleaved by Xma I and BamH I from the 55 vector pSP72-HCCl37) into the Xma I-BamHI sites of the vaccinia plasmid pvHCV-10A. The resulting plasmid was named pvHCV-37. After confirmatory sequencing, the amino-terminal region containing the internal deletion was isolated from this vector pvHCV-37 (cleavage by EcoR I and 60 BstE II) and reinserted into the Eco RI and Bst Ell-cut pvHCV-11A plasmid. This construct was expected to express an E1 protein with both hydrophobic domains deleted and was named pvHCV-38. The E1-coding region of clone HCCl38 is represented by SEQ ID NO 23.

As the hydrophilic region at the E1 carboxyterminus (theoretically extending to around amino acids 337-340)

cloned into an EcoRII/BamHI-cut pgsATA-18 vector. The resulting plasmid was named pvHCV-39 and contained clone HCCl39 (SEQ ID NO 25). The same fragment was cleaved from the pvHCV-37 vector by BamH I (of which the sticky ends were filled with Klenow DNA Polymerase I (Boehringer)) and subsequently by EcoR1 (5' cohesive end). This sequence was inserted into the EcoR1 and Bbr PI-cut vector pMS-66. This resulted in clone HCCl40 (SEQ ID NO 27) in plasmid pvHCV-40, containing a 6 histidine tail at its carboxy-terminal end.

## 2.3. E1 of Other Genotypes

Clone HCCl62 (SEQ ID NO 29) was derived from a type 3a-infected patient with chronic hepatitis C (serum BR36, clone BR36-9-13, SEQ ID NO 19 in WO 94/25601, and see also Stuyver et al. 1993a) and HCCl63 (SEQ ID NO 31) was derived from a type 5a-infected child with post-transfusion hepatitis (serum BE95, clone PC-4-1, SEQ ID NO 45 in WO 94/25601).

### 2.4. E2 Constructs

The HCV E2 PCR fragment 22 was obtained from serum BE11 (genotype 1b) by means of primers HCPr109 (SEQ ID NO 33) and HCPr72 (SEQ ID NO 34) using techniques of RNA preparation, reverse-transcription and PCR, as described in Stuyver et al., 1993b, and the fragment was cloned into the Sma I-cut pSP72 vector. Clone HCC122A (SEQ ID NO 35) was cut with Ncol/AlwNI or by BamHI/ AlwNI and the sticky ends of the fragments were blunted (Ncol and BamHI sites with Klenow DNA Polymerase I (Boehringer), and AlwNI with T4 DNA polymerase (Boehringer)). The BamHI/AlwNI cDNA fragment was then inserted into the vaccinia pgsATA-18 vector that had been linearized by EccRI and Hind III cleavage and of which the cohesive ends had been filled with Klenow DNA Polymerase (Boehringer). The resulting plasmid was named pvHCV-41 and encoded the E2 region from amino acids Met347 to Gln673, including 37 amino acids (from Met347 to Gly383) of the E1 protein that can serve as signal sequence. The same HCV cDNA was inserted into the EcoR I and Bbr Pl-cut vector pMS66, that had subsequently been blunt ended with Klenow DNA Polymerase. The resulting plasmid was named pvHCV-42 and also encoded amin acids 347 to 683. The Ncol/AlwNI fragment was inserted in a similar way into the same sites of pgsATA-18 (pvHCV-43) or pMS-66 vaccinia vectors (pvHCV-44), pvHCV-43 and pvHCV-44 encoded amino acids 364 to 673 of the HCV polyprotein, of which amino acids 364 to 383 were derived from the natural carboxyterminal region of the E1 protein encoding the signal sequence for E2, and amino acids 384 to 673 of the mature E2 protein.

2.5. Generation of Recombinant HCV-Vaccinia Viruses Rabbit kidney RK13 cells (ATCC CCL 37), human osteosarcoma 143B thymidine kinase deficient (TK-) (ATCC CRL 8303), HeLa (ATCC CCL 2), and Hep G2 (ATCC HB 8065) cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, Md., USA).



ible 4. Change of Envelope Antibody levels over time (complete study, 28 patients)

WO 96/04385

ELAB LTR All	0.0840 0.0464" 0.0058"
E2Ali NR Ali	0.0186 0.0640 0.04326 0.0464 0.0869
ETAb LTR Iypa 3a	0.063 0.0277"
ELAB LTR type 1b	0.043
E1Ab LTR All	0.0058
E1Ab NR Iype 3a	0.285 0.5930 1
ETAB NR type 1b	0.2604 0.7213 0.3105
E1Ab NR All	0.1167 0.86 0.7989
ilcoxon Signed ink tost (P values)	nd of therapy* months follow up*  ? months follow up*

Jala were compared with values obtained at initiation of thurapy P values < 0.05

TABLE 1-continued

	Recombin	ant vaccinia plasmids and	viruses	
Plasmid name	Name	cDNA subclone construction	Length (nt/aa)	Vector used for insertion
pvHCV-65	E1-E2	BamH I - Hind III	2072/691	pvHCV-10A
pvHCV-66	CORE-E1-E2	BamH I - Hind III	2427/809	pvHCV-33
pvHCV-81	E1*-GLY 1	EcoRI - BamH I	783/262	pvHCV-10A
pvHCV-82	E1*-GLY 2	EcoRI - BamH I	783/262	pvHCV-10A
pvHCV-83	E1*-GLY 3	EcoRI - BamH I	783/262	pvHCV-10A
pvHCV-84	E1*-GLY 4	EcoRI - BamH I	783/262	pvHCV-10A
pvHCV-85	E1*-GLY 5	EcoRI - BamH I	783/262	pvHCV-10A
pvHCV-86	E1*-GLY 6	EcoRI - BamH I	783/262	pvHCV-10A

nt: nucleotide
aa: aminoacid
Kl: Klenow DNA Pol filling
T4:T4 DNA Pol filling
Position: aminoacid positionin the HCV polyprotein sequence

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TABLE 2

		Summary of anti-E1 tests N ± SD (mean anti-E1 titer)			26	Syn	thetic p	eptides for competitio	n studie	6
s	Start of treatme	ent End of treatment	Follow-up		25					SEC
	i.94 ± 2.29 1:3946)	4.48 ± 2.69 (1:568)	2.99 ± 2.69 (	1:175)		PROTEIN	PEPTIDE	AMINO ACID SEQUENCE	POSITION	ID N NO
NR Š	5.77 ± 3.77 1:1607)		6.08 ± 3.73 (1:1978)		30		E1-63	VVLLLPAGVDAETIVSGGQA	373-392	71
	,				-	E2	E2-67	SGLVSLFTPGAKQNJQLINT	397-416	72
		ed response for more than 1					E2-69	QNIQLINTNGSWHINSTALN	409-428	73
NR: No re	sponse, respon	ise with relapse, or partial re	sponse				E2-\$3B	LNCNESLNTGWWLAGLIYQHK	427-446	74
							E2-\$1B	AGLIYOHKFNSSGCPERLAS	439-458	75
		TABLE 3			35		E2-1B	GCPERLASCRPLTDFDQGWG	451-470	76
		TABLE 3			-		E2-3B	TDFDQGWGPISYANGSGPDQ	463-482	77
Syr	thetic per	ptides for competiti	on studie	8			E2-5B	ANGSGPDORPYCWHYPPKPC	475-494	78
							E2-7B	WHYPPKPCGIVPAKSVCGPV	487-506	79
				SEQ						
				TD			E2-9B	AKSVCGPVYCFTPSPVVVGT	499-518	80
PROTEIN	PEPTIDE A	AMINO ACID SEQUENCE	POSITION	ID N NO	40		E2-9B E2-11B	AKSVCGPVYCFTPSPVVVGT PSPVVVGTTDRSGAPTYSWG	499-518 511-530	
				N NO	40			PSPVVVGTTDRSGAPTYSWG		81
PROTEIN	E1-31 1	LLSCLTVPASAYQVRNSTGL	181-200	56	40		E2-11B	PSPVVVGTTDRSGAPTYSWG GAPTYSWGENDTDVFVLNNT	511-530 523-542	81 82
	E1-31 1 E1-33 (		181-200 193-212	56 57	40		E2-11B E2-13B E2-17B	PSPVVVGTTDRSGAPTYSWG GAPTYSWGENDTDVFVLNNT GNWFGCTWMNSTGFTKVCGA	511-530 523-542 547-566	81 82 83
	E1-31 1 E1-33 ( E1-35 1 E1-35A 2	LLSCLTVPASAYQVRNSTGL QVRNSTGLYHVTNDCPNSS I	181-200 193-212 205-224 208-227	56 57 58	40		E2-11B E2-13B E2-17B E2-19B	PSPVVVGTTDRSGAPTYSWG GAPTYSWGENDTDVFVLNNT GNWFGCTWMNSTGFTKVCGA GFTKVCGAPPVCIGGAGNNT	511-530 523-542 547-566 559-578	81 82 83
	E1-31 1 E1-33 ( E1-35 1 E1-35A 5 E1-37 E	LLSCLTVPASAYQVRNSTGL QVRNSTGLYHVTNDCPNSS I NDCPNSSIVYEAHDAILHTF SNSSIVYEAADMIMHTPGCV HDAILHTPGCVPCVREGNVS	181-200 193-212 205-224 208-227 217-236	56 57 58 59 60			E2-11B E2-13B E2-17B E2-19B E2-21	PSPVVVGTTDRSGAPTYSWG GAPTYSWGENDTDVFVLNNT GNWPGCTWMNSTGFTKVCGA GFTKVCGAPPVCIGGAGNNT IGGAGNNTLHCPTDCFRKHP	511-530 523-542 547-566 559-578 571-590	81 82 83 84 85
	E1-31 1 E1-33 ( E1-35 1 E1-35A 2 E1-37 E1-39 (	LLSCLTVPASAYQVRNSTGL QVRNSTGLYHVTNDCPNSSI NDCPNSSIVYEAHDAILHTF SNSSIVYEAADMIHHTPGCV HDAILHTPGCVPCVREGNVS CVREGNVSRCWVAMTPTVAT	181-200 193-212 205-224 208-227 217-236 229-248	56 57 58 59 60 61	40 45		E2-11B E2-13B E2-17B E2-19B E2-21 E2-23	PSPVVVGTTDRSGAPTYSWG GAPTYSWGENDTDVFVLNNT GNWPGCTWMNSTGFTKVCGA GFTKVCGAPFVCIGGAGNNT IGGAGNNTLHCPTDCFRKHP TDCFRKHPDATYSRCGSGFW	511-530 523-542 547-566 559-578 571-590 583-602	81 82 83 84 85 86
	E1-31 1 E1-33 ( E1-35 1 E1-35A 2 E1-37 E E1-39 (	LLSCLTVPASAYQVRNSTGL QVRNSTGLYHVTNDCPNSS I NDCPNSSTVYEAHDAILHTP SNSSTVYEAADMIMHTPGCV HDAILHTPGCVPCREGNVS CVREGNVSRCWVAMTPTVAT AMTPTVATRDGKLPATQLRR	181-200 193-212 205-224 208-227 217-236 229-248 241-260	56 57 58 59 60 61 62			E2-11B E2-13B E2-17B E2-19B E2-21 E2-23 E2-25	PSPVVVGTTDRSGAPTYSWG GAPTYSWGENDTDVFVLNNT GNWPGCTWMNSTGFTKVCGA GFTKVCGAPFVCIGGAGNNT IGGAGNNTLHCPTDCFRKHP TDCFRKHPDATYSRCGSGFW	511-530 523-542 547-566 559-578 571-590	81 82 83 84 85
	E1-31 II E1-33 ( E1-35 II E1-35A II E1-37 II E1-39 ( E1-41 II E1-43 II	LLSCLTVPASAYQVRNSTGL QVRNSTGLYHVTNDCPNSS I NDCPNSSIVYEAHDATLHTP SNSSIVYEAADMIMHTPGCV HDAILHTPGCVPCVREGNVS CVREGNVSRCWVAMTPTVAT AMTPTVATRDGKLPATQLRR LPATQLRRHIDLLVGSATLC	181-200 193-212 205-224 208-227 217-236 229-248 241-260 253-272	56 57 58 59 60 61 62 63			E2-11B E2-13B E2-17B E2-19B E2-21 E2-23	PSPVVVGTTDRSGAPTYSWG GAPTYSWGENDTDVFVLNNT GNWPGCTWMNSTGFTKVCGA GFTKVCGAPFVCIGGAGNNT IGGAGNNTLHCPTDCFRKHP TDCFRKHPDATYSRCGSGFW	511-530 523-542 547-566 559-578 571-590 583-602 595-614	81 82 83 84 85 86
	E1-31 II E1-33 (II E1-35 II E1-35A II E1-37 II E1-39 II E1-41 II E1-43 II	LLSCLTVPASAYQVRNSTGL QVRNSTGLYHVTNDCPNSS I NDCPNSSTVYEAHDAILHTP SNSSTVYEAADMIMHTPGCV HDAILHTPGCVPCREGNVS CVREGNVSRCWVAMTPTVAT AMTPTVATRDGKLPATQLRR	181-200 193-212 205-224 208-227 217-236 229-248 241-260 253-272 265-284	56 57 58 59 60 61 62			E2-11B E2-13B E2-17B E2-19B E2-21 E2-23 E2-25	PSPVVVGTTDRSGAPTYSWG GAPTYSWGENDTDVFVLNNT GNWPGCTWMNSTGFTKVCGA GFTKVCGAPPVCIGGAGNNT IGGAGNNTLHCPTDCFRKHP TDCFRKHPDATYSRCGSGPW SRCGSGPWITPRCLVDYPYR	511-530 523-542 547-566 559-578 571-590 583-602 595-614	81 82 83 84 85 86
	E1-31 E1-33 E1-35 E1-35A E1-37 E1-39 E1-41 E1-43 E1-45 E1-49 (61-49)	LLSCLTVPASAYQVRNSTGL QVRNSTGLYHVYNDCPNSSI NDCPNSSIVYEAHDAILHTF SNSSIVYEAADMIMHTPGCV BOALLHTPGCVPCVREGOVS CVREGNVSRCWVAMTPTVAT AMTPTVATTAGKLPATQLER LPATQLRRHIDLLVGSATLC LVGSATLCSALYVGDLCGSV	181-200 193-212 205-224 208-227 217-236 229-248 241-260 253-272 265-284 289-308	56 57 58 59 60 61 62 63 64	45		E2-11B E2-13B E2-17B E2-19B E2-21 E2-23 E2-25 E2-27	PSPVVVGTTDRSGAPTYSWG GAPTYSWGENDTDVFVLNNT GNWFGCTWMNSTGFTKVCGA GFTKVCGAPPVCIGGAGNNT IGGAGNNTLHCPTDCFRKHP TDCPRKHPDATYSRCGSGPW SRCGSGPWITPRCLVDYPYR CLVDYPYRLWHYPCTNYTI PCTINYTIFKIRMYVGGVEH	511-530 523-542 547-566 559-578 571-590 583-602 595-614 607-626	81 82 83 84 85 86 87 88
	E1-31 E1-33 E1-35 E1-35A E1-37 E E1-37 E E1-49 E1-49 E1-49 E1-49 E1-53 E1-53	LLSCLTVPASAYQVRNSTGL QVRNSTGLYHVYNDCPNSSI NDCPNSSIVYEAHDAILHTF SNSSIVYEAADMIMHTPGCV HOAILHTPGCVPCVREGNVS CVRECNVSRCWVAMTPTVATA MTPTVATRDGKLPATQLRR LPATQLRRHIDLLVGSATLC QUSSATLCSALVVGDLCGSV QLFTFSPRRHWTTQGCNCSI	181-200 193-212 205-224 208-227 217-236 229-248 241-260 253-272 265-284 289-308 301-320	56 57 58 59 60 61 62 63 64 65			E2-11B E2-13B E2-17B E2-19B E2-21 E2-23 E2-25 E2-27 E2-29 E2-31	PSPVVVGTTDRSGAPTYSWG GAPTYSWGENDTDVPVLNNT GNWFGCTWMNSTGFTKVCGA GFTKVCGAPPVCIGCAGNNT IGGAGNNTLHCPTDCFRKHP TDCPRKHPDATYSRCGSGPW SRCGSGPWITPRCLVDYPYR CLVDYPYRLWHYPCTNYTI PCTINYTIFKIRMYVGGVEH MYVGGVEHRLEAACNWTPGE	511-530 523-542 547-566 559-578 571-590 583-602 595-614 607-626 619-638 631-650	81 82 83 84 85 86 87 88 89
	E1-31 I E1-35 I E1-35 I E1-37 I E1-39 (I E1-43 I E1-45 I E1-45 I E1-51 I E1-51 I E1-55 I	LLSCLTVPASAYQVRNSTGL QVRNSTGLYHVTNDCPNSS I NDCPNSS IVYEANDAILHTP SNSS IVYEANDMIMHTPGCV HDAILHTPGCVPCVREGNVS CVREGNVSRCWVAMTPTVAT AMTPTVATRDGKLPATQLER LPATQLERHIDLLVGSATLC LVGSATLCSALYVGDLCGSV QLPTFSPRRHWTTQGCNCS I QGGNCS IVPGHITGHRMAW	181-200 193-212 205-224 208-227 217-236 229-248 241-260 253-272 265-284 289-308 301-320 313-332 325-344	56 57 58 59 60 61 62 63 64 65 66	45		E2-11B E2-13B E2-17B E2-19B E2-21 E2-23 E2-25 E2-27 E2-29	PSPVVVGTTDRSGAPTYSWG GAPTYSWGENDTDVPVLNNT GNWFGCTWMNSTGFTKVCGA GFTKVCGAPPVCIGGAGNNT IGGAGNNTLHCPTDCFRKHP TDCPRKHPDATYSRCGSGPW SRCGSGFWITPRCLVDYPYR CLVDYPYRLWHYPCTNYTI PCTINYTIFKIRMYVGGVEH MYVGGVEHRLEAACNWTPGE ACNWTPGERCDLEDRDRSEL	511-530 523-542 547-566 559-578 571-590 583-602 595-614 607-626 619-638	81 82 83 84 85 86 87 88 89 90

TA	DI	$\mathbf{C}$	,

	Change	of Envelope	Antibody lev	els over time	(complete stud	y, 28 patients)		
Wilcoxon Signed Rank test (P values)	E1Ab NR All	E1Ab NR type 1b	E1Ab NR type 3a	E1Ab LTR II	E1Ab LTR type 1b	E1Ab LTR type 3a	E2Ab NR All	E1Ab LTR Ail
End of therapy*	0.1167	0.2604	0.285	0.0058**	0.043**	0.0499**	0.0186**	0.0640
6 months follow up*	0.86	0.7213	0.5930	0.0047**	0.043**	0.063	0.04326	0.0464**
12 months follow up*	0.7989	0.3105	1	0.0051**	0.0679	0.0277**	0.0869	0.0058**

<sup>\*</sup>Data were compared with values obtained at initiation of therapy

\*\*P values < 0.05

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Blec: 09/12/20/-

# CODING SAOEZEGO

		• •		
		Average SIN 2.495223 2.902185 2.587447 4.279076 2.886046 2.555075 3.109195		Averago E VGLY# 0.0006815 0.903077 0.799967 1.51608 0.907783
		Sum SN 59.88534 69.65243 62.09872 102.6978 69.26511 61.32181		Sum E 1/GLY# 19.36524 21.67304 19.19921 36.38592 21.78679 19.59691
	1.629403 2.070524 1.721164 3.955153 2.07278 1.744221 2.593066	24 1.706992 1.632785 1.20376 2.481595 1.638211 1.716423	12 0.628171 0.798232 0.663547 1.524798 0.799102	24 0.957620 0.915998 0.675314 1.392178 0.919042
	11 1,220654 1,467582 1,464216 4,250784 1,562092 1,529608 1,55719	23 2.150089 1.661914 1.336775 3.68213 1.817901 1.475062 2.083333	11 0.703002 0.942455 0.940294 2.72970 1.003140	23 0.797719 0.641652 1.767422 0.872593
	10 2.468162 2.482212 2.191558 5.170841 3.021807 2.677757 2.616822	22 1.180746 1.150761 0.97767 2.393011 1.153656 1.280743 1.167286	10 0.94319 0.94656 0.637466 1.976 1.154762	22 1.010386 0.98586 0.837550 2.050064 0.986323
	9 1.730193 1.688973 1.602222 3.710507 1.708937 1.704976	21 4.378633 4.680101 4.268633 4.293038 4.64557 2.781063 5.35443	9 0.958261 0.935431 0.887385 2.05505 0.946489	21 0.817759 0.874061 0.797215 0.801773 0.067612 0.519395
	1.866103 1.595477 1.482099 3.959542 1.576336 1.496489	20 2.47171 2.921288 2.557384 3.002535 3.126761 2.665433 3.678068	0.954961 0.016436 0.758410 2.026172 0.606641	20 0.672013 0.794245 0.695306 0.850109 0.724683
:LISA	7 1.950345 2.146302 1.96692 4.196751 2.13912 2.02069 2.287753	19 1.93476 2.127712 1.980185 3.013321 2.442804 1.506716	7 0.052510 0.93017 0.059761 1.035317 0.935031	19 0.698162 0.76779 0.714554 1.376045 0.881491 0.543702
nts.by_E	2.866913 5.043993 4.833742 4.71302 4.964765	18 6.675179 7.65433 5.775357 6.4125 5.424107 5.191964	6 0.588794 1.035913 0.992733 0.967939 1.019642	10 0.928144 1.064269 0.803029 0.89162 0.75419
on_muta	2.120191 2.459019 1.591010 3.15 1.715311 2.494833 3.131579	2.317721 2.933792 2.515305 5.604613 2.654224 2.363301 2.980354	5 0.677036 0.785233 0.508312 1.005882 0.547746	0.777666 0.984377 0.843962 1.080587 0.890574
cosylatic	4 1.205597 2.639308 2.354748 1.499307 2.627358 2.527925 2.790801	16 1.985105 3.055649 2.945628 5.684498 3.338912 2.572385 3.280335	4 0.431977 0.94569 0.84373 0.537245 0.941408 0.90578	16 0.605153 0.931505 0.697966 1.732902 1.017857 0.784184
f.E.L.gly	3 1.403871 2.325495 2.251648 3.874605 2.409344 2.131613	15 3.763498 3.621928 3.016099 5.707668 3.125561 2.621704	3 0.55869 0.925463 0.900053 1.541952 0.958831	1526988 1.180833 0.983319 1.860833 1.019006 0.854737
nalysis_o	2.120971 1.76816 1.715477 3.824038 1.793761 1.495737	14 3.233604 2.567613 2.763055 6.581122 2.940334 2.499952 3.183771	2 0.952374 0.793961 0.770296 1.717097 0.605447	1,015852 0.006469 0.067856 2.060802 0.923538
Table B. Analysis of F.1 glycosylation mutants by FilsA serum	1.002462 2.400795 1.642710 2.570154 2.402051 2.031407	5.005561 7.556602 7.930530 0.176816 8.003400 8.005561	SERUM 1 0.637316 0.048876 0.560834 0.911587 0.077607	0.644248 0.85627 0.098633 0.92654 1.006606
r.	1.Y1 1.Y2 1.Y3 1.Y3 1.Y4 1.Y5 1.Y6	11 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	<u> </u>	<u> </u>

TARLE 8

			S	SERUM							
	4	s.	٥	7	80	6	10	=	12		
1.403871 2.325495 2.261646 3.874605 2.409344 2.131613 2.512792	1.205597 2.639308 2.354748 1.499387 2.627358 2.527925 2.790881	2.120191 2.459019 1.591818 3.15 1.715311 2.49433 3.131579	2.866913 5.043993 4.833742 4.71302 4.964765 4.784027	1.950345 2.146302 1.96692 4.198751 2.13912 2.02069 2.287753	1.866183 1.595477 1.482099 3.959542 1.576336 1.496489	1.730193 1.688973 1.602222 3.710507 1.708937 1.704976 1.805556	2.468162 2.482112 2.191558 5.170841 3.021807 2.677757	1.220654 1.467582 1.464216 4.250784 1.562092 1.529608 1.55719	1.629403 2.070524 1.721164 3.955153 2.07278 1.744221 2.593886		
15	16	17	18	19	20	21	22	23	Sum 24	Average S/N	S/N
3.233684 3.763498 2.567613 3.621928 2.763055 3.016099 6.561122 5.707668 2.940334 3.125561 3.183771 3.067265	1.985105 3.055649 2.945628 5.684498 3.338912 2.572385 3.280335	2.317721 2.933792 2.515305 5.604813 2.654224 2.363301 2.980354	6.675179 7.65433 5.775357 6.4125 5.424107 5.194107	1,93476 2,127712 1,980185 3,813321 2,442804 1,586716 2,771218	2.47171 2.921288 2.557384 3.002535 3.126761 2.665433 3.678068	4.378633 4.680101 4.268633 4.293038 4.64557 2.781063 5.35443	1.188748 1.158781 0.97767 2.393011 1.153656 1.280743	2.158889 1.661914 1.336775 3.68213 1.817901 1.475862 2.083333	1.786992 1.632785 1.20376 2.481585 1.638211 1.716423	59.88534 69.65243 62.89872 102.6978 69.26511 76.54068	2.495223 2.982185 2.587447 4.279076 2.886846 2.555075 3.189195
			IS.	ERUM							
3	4	S.	9	7	80	٥	10	11	12		
0.952374 0.55869 0.793961 0.925463 0.770296 0.900053 11.717097 1.541952 0.805447 0.958831 0.671626 0.848305	0.431977 0.94569 0.84373 0.537245 0.941488	0.677036 0.785233 0.508312 1.005882 0.547746 0.796669	0.588794 1.035913 0.992733 0.967939 1.019642 0.982522	0.852516 0.93817 0.859761 1.835317 0.935031 0.883264	0.954961 0.816436 0.758418 2.026172 0.806641 0.765781	0.958261 0.935431 0.887385 2.05505 0.946488 0.944294	0.94319 0.94856 0.837488 1.976 1.154762	0.783882 0.942455 0.940294 2.72978 1.883148 0.982288	0.628171 8.798232 8.663547 1.524798 8.799182 8.672435		
15	16	17	18	19	20	21	22	23	24	Sum E1/GLY #	Average E1/GLY #
1.815652 1.22698 6.806469 1.188833 0.867856 8.983319 2.060802 1.860833 0.923538 1.019006 0.785217 8.854737	0.605153 0.931505 0.897966 1.732902 1.817857 0.784184	0.777666 8.984377 0.843962 1.880587 0.890574 0.79296	0.928144 1.064289 0.883029 0.89162 8.75419 8.72221	0.698162 8.76779 0.714554 1.376045 0.881491 0.543702	0.672013 0.794245 0.695306 8.816335 0.850109 8.724683	0.817759 0.874061 0.797215 8.801773 8.867612 0.519395	1.018386 0.98586 0.837558 2.058864 8.988323 1.097197	1.836267 8.797719 0.641652 1.767422 0.872593 0.70803	0.957628 8.915998 0.675314 1.392178 0.919042 0.962919	19.36524 21.67384 19.19921 36.38592 21.78679 19.59691	0.806885 0.903077 0.799967 1.51608 8.907783 0.816538
		2.261646 2.261646 2.874605 2.40344 2.131613 2.512792 3.621928 3.016699 5.707668 3.016699 5.707668 3.016699 5.707668 3.0155861 2.621792 3 0.55869 0.958831 0.848305 1.541952 0.898833 0.848319 1.8608333 1.0190006 8.854737 0.120190006	2.561646 2.554748 2.69347 4.99387 2.49346 2.554748 2.112613 2.277925 2.112792 2.790881 1.5 16 16 16 16 16 16 16 16 16 16 16 16 16	2.56.196         2.60.306         2.60.3078         2.50.3078	2.561646 2.354748 1.591818 4.833742 2.8016466 2.354748 1.591818 4.833742 2.409344 2.627358 1.1591818 4.833742 2.409344 2.627358 1.1591818 4.833742 2.409344 2.627358 1.1715311 4.964765 2.131613 2.527952 2.494833 4.78407 2.627192 3.623192 2.363291 2.313721 6.675179 2.6272182 2.363391 6.4125 3.105699 2.945628 2.515367 2.515367 2.624224 6.4125 3.105699 2.945628 2.515367 2.624224 6.4125 3.1067266 3.280335 2.980354 7.191964 2.572385 2.64321 2.594313 0.992343 0.092322 0.092344 0.092343 0.092343 0.092343 0.092343 0.092343 0.092343 0.092343 0.092343 0.092	25616-66 2.3547-88 1.591818 4.8337-92 2.144502. 25616-66 2.3547-88 1.591818 4.8337-92 2.144502. 25127924 2.25773-88 1.715311 4.964762 2.13912 1.240934 2.2577925 2.494633 4.784677 2.02092 2.131613 2.2277925 2.494633 4.784677 2.02092 2.131613 2.2277925 2.494633 4.784677 2.02092 2.131613 2.2277925 2.494633 4.784677 2.02092 2.045628 2.317721 6.675179 1.93476 2.287753 1.31556 3.328033 2.2933792 7.65433 1.227712 2.2277068 2.69498 5.604913 6.4125 3.813321 3.31556 3.328033 2.980354 7.191964 2.771218 3.31556 3.3280335 2.980354 7.191964 2.771218 3.067265 3.280335 2.980354 7.191964 2.771218 3.261709 0.925463 0.431977 0.677036 0.58879 0.858791 0.092543 0.598791 0.092543 0.598791 0.092543 0.598791 0.992543 0.598791 0.992543 0.992954 0.99296 0.992996 0.992996 0.992999 0.99299 0.99299 0.99299 0.99299 0.99299 0.99299 0.99299 0.99299 0.99299 0	2.561646 1.492987 1.3518 1.352772 2.105092 1.325471 1.2561646 1.492987 1.315 4.71302 1.361645 1.452099 1.31518 1.31518 1.31318	25616-66         2354748         1292017         240202         1202017 <t< td=""><td>2561666         2.354748         2.473073         2.173073         2.173071         2.173071         2.482079         2.492121         2.492121         2.492373         2.171020         2.173071         2.492373         2.171020         2.170071</td><td>15.261.79         1.50.01.00         1.50.01.</td><td>2.5.15.45.8         2.5.25.48         1.5.25.48         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.7.25.49         1.5.25.79         1.5.25.79         1.7.25.79         1.5.25.79         1.7.25.79         1.5.25.79         1.7.25.79</td></t<>	2561666         2.354748         2.473073         2.173073         2.173071         2.173071         2.482079         2.492121         2.492121         2.492373         2.171020         2.173071         2.492373         2.171020         2.170071	15.261.79         1.50.01.00         1.50.01.	2.5.15.45.8         2.5.25.48         1.5.25.48         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.5.25.49         1.7.25.49         1.5.25.79         1.5.25.79         1.7.25.79         1.5.25.79         1.7.25.79         1.5.25.79         1.7.25.79

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Table 5. Difference between LTR and NR (complete study)

M.O 36/01382

Mann-Withney U test (P values)	EIAb S/N Ail	E1Ab illers E1Ab S/N All type 1b	EIAb S/N type 1b	E1Ab S/N 1 type 3a /	E2Ab S/N Ali
Initiation of therapy End of therapy	0.0257		0.05	0.68	0.1078
6 months follow up. 12 months follow up	0.67		0.6099 0.23	0.425 0.4386	0.3081 0.6629

P values < 0.05

TOOLOT " SZOEZGGO

able 6. Compatition experiments between muring E2 monocland muliberiles

	12D11F1 15C8C1 8G10D1H9	
	15CBC1	
	12011F1	
	963E6	r.
nabs	17C2F2	S
anti-E2 n	4H6B2	-
lotinylated	1003C4	QN
ctivity of b	16A6E7	10
anti-E2 reac	2F10H10	62
Dacrenso (%) of anti-E2 reactivity of biotinylated anti-E2 mabs	17H10F4D10 2F10H10 16A6E7 10D3C4 4H6B2 17C2F2 9G3E6	
	ompetitor	7H10F4D10

7H10F4D10	. 0	62	10	Q	=	Q	ឆ	9	30	S		
F10H10	90		-	Q	30	Q	c	~	12	Z		
0A8E7	QN	Q	•	Q	CN	CN	GN	ON	QN	Q		-
0D3C4	Ξ	50	92	•	94	26	28	43	53	9 6		
11682	CN	S	82	CN	· ,·	S	Q	Q	QN	9 2		
7C2F2	2	Q	75	QN	99		Ξ	01	C	. 0		
G3E8	Q	QN	89	Q	Ξ	C		09	76			
2D11F1	QN	Q Q	. 26	Q	13	Q	QN		88	Q		
5C8C1	Q	Q	18	Q	01	QV	Q	Ç		2		
G10D1H9	2	2	=	Q	15	Q	67	082	81			
ampatitor controls	ontrols											
587A2 H6A7 3C12H9	O O O	0 8 N	60.	15.	0 0 QN	60 4	0 0 CN	. 0 4 S	. c o 2	15 O S	:	

ID, not dong

WO 96/04385

## FOOTER' WHORKARD

## Table 7. Primer

SEQ ID NO. 96	GPT	5'-GTTTAACCACTGCATGATG.3'
SEQ ID NO. 97	ΤK <sub>n</sub>	5'-GTCCCATCGAGTGCGGCTAC-3'
SEO ID NO. 98	GLY1	5'-CGTGACATGGTACAT <u>TCCGGA</u> CACTTGGCGCACTTCATAAGCGGA-3'
SEQ ID NO. 99	GLY2	5'-TGCCTCATACACAATG <u>GAGCTC</u> TGGGACGAGTCGTTCGTGAC-3'
SEQ ID NO. 100	GLY3	5'-TACCCAGCAGCGGAGCTCTGTTGCTCCCGAACGCAGGGCAC.3'
SEQ 1D NO. 101	GLY4	5'-TGTCGTGGGGACGQ <u>AGGCCT</u> GCCTAGCTGCGAGCGTGGG-3'
SEQ ID NO. 102	GLY5	5'-CGTTATGTGG <u>CCCGGG</u> TAGATTGAGCACTGGCAGTCCTGCACCGTCTC-3
SEQ 1D NO. 103	GLY6	5'-CAGGGCCGTTGT <u>AGGCCT</u> CCACTGCATCATATCCCAAGC-3'
SEQ ID NO. 104	OVR1	5'- <u>CCGGA</u> ATGTACCATGTCACGAACGAC-3'
SEQ ID NO. 105	OVR2	5'- <u>gcic</u> cattgtgtatgaggcagcgg.3'
SEQ ID NO. 106	OVR3	5'- <u>GAGCTC</u> CCGCTGCTGGGTAGCGC-3'
SEQ ID NO. 107	OVR4	5'. <u>cci</u> ccgiccccaccacaatacg.3'
SEQ ID NO. 108	OVR5	5'-CTA <i>CCCGG</i> GCCACATAACGGGTCACCG-3'
SEQ ID NO. 109	OVR6	5'-GG <u>AGGCCT</u> ACAACGGCCCTGGTGG-3'
SEQ ID NO. 110	GPT-2	5'-TTCTATCGATTAAATAGAATTC .3'
SEO ID NO. 111	TKn-2	5'-GCCATACGCTCACAGCCGATCCC.3'

nucleotides in bold represent mutations with respect to the original HCC110A sequence

nucleotides underlined represent additional restriction site

TABLE 5

	17	ADLE 3	,									
Difference  Mann-Withney	E1Ab S/N	TR and N	IR (complete E1Ab S/N	E1Ab	E2Ab S/N	5	content of page 69					
U test (P values)	All	all	type 1b	type 3a	All							
Initiation of therapy End of therapy	0.0257° 0.1742		0.05*	0.68	0.1078 0.1295	10						
6 months follow up,	1		0.6099	0.425	0.3081							
12 months follow up	0.67		0.23	0.4386	0.6629							

<sup>\*</sup>P values < 0.05

TABLE 6

				IMBLI	- 0					
	Com	petition exp	eriments b	etween m	urine E2	monoclon	al antibod	ies		
	Decrease (%) of anti-E2 reactivity of biotinylated anti-E2 mabs									
	17H10F4D10	2F10H10	16A6E7	10D3C4	4H6B2	17C2F2	9G3E6	12D11F1	15C8C1	8G10D1H9
mpetitor								-		
H10F4D10		62 .	10	ND	11	ND	5	6	30	ND
710H10	90		1	ND	30	ND	0	4	12	ND
6A6E7	ND	ND	_	ND	ND	ND	ND	ND	ND	ND
D3C4	11	50	92	_	94	26	28	43	53	30
16B2	ND	ND	82	ND	_	ND	ND	ND	ND	ND
C2F2	2	ND	75	ND	56	· —	11	10	0	0
33E6	ND	ND	68	ND	11	ND	_	60	76	ND
D11F1	ND	ND ND	26	ND	13	ND	ND	_	88	ND
5C8C1	ND	ND	18	ND	10	ND	ND	ND	_	ND
G10D1H9 empetitor controls	2	2	11	ND	15	ND	67	0.82	81	_
5B7A2	0	0	9	15	10	9	0	0	0	5
H6A7	0	2	0	12	8	0	0	4	0	0
23C12H9	ND	ND	2	12	ND	4	ND	ND	ND	2

ND, not done

TABLE 7

					Primers
SEQ	ID	NO.	96	GPT	5'-GTTTAACCACTGCATGATG-3'
SEQ	ID	NO.	97	TK <sub>R</sub>	5'-GTCCCATCGAGTGCGGCTAC-3'
SEQ	ID	NO.	98	GLY1	5'-CGTGACATGGTACATTCCGGACACTTGGCGCACTTCATAAGCGGA-3'
SEQ	ID	NO.	99	GLY2	5'-TGCCTCATACACAATGGAGCTCTGGGACGAGTCGTTCGTGAC-3'
SEQ	ID	NO.	100	GLY3	5'-TACCCAGCAGCGGGAGCTCTGTTGCTCCCGAACGCAGGGCAC-3'
SEQ	ID	NO.	101	GLY4	5'-TGTCGTGGTGGGACGGAGGCCTGCCTAGCTGCGAGCGTGGG-3'
SEQ	ID	NO.	102	GLY5	5'-CGTTATGTGGCCCGGGTAGATTGAGCACTGGCAGTCCTGCACCGTCTC-3
SEQ	ΙD	NO.	103	GLY6	5'-CAGGGCCGTTGTAGGCCTCCACTGCATCATCATATCCCAAGC-3'
SEQ	ID	NO.	104	OVR1	5'-CCGGAATGTACCATGTCACGAACGAC-3'
SEQ	ID	NO.	105	OVR2	5'-GCTCCATTGTGTATGAGGCAGCGG-3'
SEQ	ID	NO.	106	OVR3	5'-GAGCTCCCGCTGCTGGGTAGCGC-3'
SEQ	ID	NO.	107	OVR4	5'-CCTCCGTCCCCACCACGACAATACG-3'
SEQ	ID	NO.	108	OVR5	5'-CTACCCGGGCCACATAACGGGTCACCG-3'
SEQ	ID	NO.	109	OVR6	5'-GGAGGCCTACAACGGCCCTGGTGG-3'
SEQ	ID	NO.	110	GPT-2	5'-TTCTATCGATTAAATAGAATTC-3'
SEQ	ID	NO.	111	TK <sub>R</sub> -2	5'-GCCATACGCTCACAGCCGATCCC-3'

nucleotides underlined represent additional restriction site nucleotides in bold represent mutations with respect to the original HCC110A sequence

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Barguelli Jany

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